GLUTATHIONE TRANSFERASE GENE
POLYMORPHISM IN END STAGE RENAL DISEASE

POLIMORFIZAM GLUTATION TRANSFERAZA U TERMINALNOJ BUBREŽNOJ SLABOSTI

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ABSTRACT

Chronic kidney disease is described as a progressive and irreversible deterioration in kidney function. When there is less than 10% of nephron function pertained, patients face end-stage renal disease, where renal replacement therapy is needed. Data show that the most common method used to treat advanced and permanent kidney failure is haemodialysis. Increased oxidative stress is a hallmark of end-stage renal disease (ESRD). Glutathione S-transferases (GST) are involved in the detoxification of xenobiotics and protection of oxidative damage. The role of genetic polymorphism of antioxidant enzymes GSTA1, GSTM1, GSTP1 and GSTT1 in susceptibility towards end-stage renal disease development has become prominent recently. Furthermore, GST gene polymorphism may modulate the degree of oxidative stress byproducts in end-stage renal disease patients and, therefore, influence their overall and cause-specific cardiovascular mortality.
INTRODUCTION

Chronic kidney disease is of major concern all over the world. It is estimated that in U.S. of America, around 10% of adults are suffering from chronic kidney disease (1). Data are showing that incidence of ESRD is progressively growing worldwide in the last twenty years, with more than two million affected individuals and over half million in the Europe. According to national dialysis register, there are almost 6000 people in Serbia treated with some type of renal replacement therapy (2). Incidence of ESRD in Serbia was also growing in the last decade, rising from 107 to 189 patients per million individuals. It is important to note that Serbia is among the European countries with higher incidence of CKD and ESRD.

The main causes of CKD include: diabetes mellitus, hypertension and glomerulonephritis. These three conditions are responsible for more than half of all CKD cases. Among other causes that may affect the renal function are hereditary diseases (polycystic kidney disease), malformations that occur during fetal development and lupus are described. Obstructions (kidney stones, tumors or an enlarged prostate gland in men) and repeated urinary infections are also among common reasons. There are at least two reasons for CKD to be considered as a serious condition: the first one is that these patients are at greater risk to develop cardiovascular complications, tumors and chronic infections; the second one is that majority of CKD patients will develop ESRD, the fifth stage of CKD, where renal replacement therapy is needed. Data have shown that total and cardiovascular mortality in ESRD patients is increased up to 30 fold as compared to general population (3).

Chronic kidney disease (CKD) belongs to the most well characterized oxidative diseases, in terms of the role that free radicals play in its pathogenesis, progression and complications (4,5). Various causes of CKD such as hypertension, diabetes and inflammation (e.g. glomerulonephritis) activate molecular pathways, leading to increased free radical production in the kidneys. Increased ROS production in renal tissue, as described in an elegant review of Vaziri et al.(6), is primarily driven by activation and up-regulation of ROS-producing enzymes, including NAD(P)H oxidase (NOX) isozymes, cyclooxygenase-2, lipooxygenase, as well as uncoupled nitric oxide synthase (NOS), mitochondrial dysfunction and endoplasmic reticulum stress (7). Oxidative changes, at molecular and cellular level in the kidney, are accompanied by progressive deterioration of renal function, which occurs through four clinical stages and finally results in renal failure and uremic state, often referred as end stage renal disease (ESRD) (Stage 5). In the course of CKD progression, local oxidative stress in kidneys exerts gradually to systemic level. Namely, impaired renal function leads to accumulation of toxic end-products of exogenous and endogenous origin, which pose a significant electrophilic stress and also enhance pro-oxidant condition. Having reached ESRD, patients require renal replacement therapy, performed either as regular peritoneal or hemodialysis procedures, known to be associated with leukocyte activation, leading to progressive increase in free radical production. Systemic oxidative stress in ESRD is considered to be the cornerstone of atherosclerotic process, which leads to various cardiovascular complications in these patients (6). Despite well described link of oxidative stress with cardiovascular complications in dialysis patients, the role of genetic predisposition in enhanced oxidative damage and consequent worsening of ESRD patients’ prognosis remains elusive. Among potential genetic biomarkers of susceptibility to oxidative stress and cardiovascular complications in ESRD, polymorphisms in genes encoding antioxidant enzymes glutathione transferases has emerged recently.

COMMON GST POLYMORPHISMS

Glutathione S-transferases (GSTs) (EC 2.5.1.18) are superfamily of enzymes belonging to phase II enzymes that play an important role in the detoxification reactions (8). GSTs catalyze the conjugation of the tri-peptide glutathione (GSH, γ-glutamlycysteinyl-glycine) with a hydrophobic co-substrate possessing an electrophilic center, in order to produce an inactive hydrophilic conjugate, which is easily excreted from the body. Apart from conjugation with glutathione, GSTs possess non-catalytic activity and the most important one described so far are the role of GSTP1 and GSTM1 in the inhibition of c-Jun N terminal kinase (JNK)(9).

The GST genes, located on different chromosomes, are described as highly polymorphic (10,11). Because of their well known xenobiotic-metabolizing activity, genetic variations in GST genes have gained much of the attention recently. Any change in the expression of GST protein level might possibly influence an individual’s susceptibility to carcinogens and various diseases. The GSTA1 gene is found on chromosome 6p12. The product of this gene, GSTA1 enzyme, catalyzes the conjugation of glutathione with many electrophilic compounds and possess glutathione-dependent (selenium-independent) peroxidase activity. Three linked base substitutions at positions -567, -69 and -52 are described regarding GSTA1 gene polymorphism. These substitutions result in two alleles hGSTA1*A (-52G, -69C, -567T) and hGSTA1*B (-52A, -69T, -567G) (12). Distribution of alleles varies within population. In Caucasians, around 38% possess GSTA1*A/A genotype, 48% GSTA1*A/B genotype and 14% GSTA1*B/B genotype (13). Individuals possessing GSTA1*B/B genotype are usually referred as GSTA1-low activity subjects.

The GSTM1 gene is located on chromosome 1p13.3 (14). One of the most studied polymorphisms of GSTM1 gene is a deletion polymorphism which results in a completely absent enzyme activity. The GST mu class has been recognized to metabolize carcinogens from cigarette smoke such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (15). About 50% of the Caucasians
are lacking GSTM1 activity and are, therefore, referred as GSTM1-null genotype individuals (16).

Gene for GSTP1 is located on chromosome 11q13. The most well described GSTP1 gene polymorphism represents a result of transition from Ile to Val at codon 105, a polymorphism that is known as rs1695 or GSTP1 Ile105Val (17,18). Most of the substrates for GSTP1 protein are different diol epoxides, polycyclic aromatic hydrocarbon (PAHs) found in a cigarette smoke. Therefore, polymorphic expression of GSTP1 has been linked so far with various cancer developments (19–21). In healthy, white population, the frequencies of the genotype variants of GSTP1 rs1695 polymorphism are *Ile/*Ile 51.5%, *Ile/*Val 39.4% and *Val/*Val are 9.1%, (22).

The human GSTT1 gene like GSTM1, most commonly exhibits deletion polymorphisms (23). Homozygous deletion of both GSTT1 alleles represents an GSTT1-null genotype, which is mostly associated with development of urinary bladder carcinoma, in individuals exposed to pesticides (24). Heterozygotes, with only one allele absent, may possess lower enzyme activity and are called GSTT1-active genotype carriers. Homozygous deletion of GSTT1 gene is present in about 20% of Caucasians (4).

Genetic polymorphism that affects GST expression results in alteration of enzyme activity profile. Therefore, it is reasonable to expect that individual’s antioxidant capacity will differ, depending on particular GST genotype and phenotype. From the clinical point of view, deletion and single nucleotide polymorphism of the GSTA1, GSTM1, GSTP1, and GSTT1 genes have been investigated in both malignant and non-malignant disease development. Homozygous deletion of GSTM1 gene results in absent GSTM1 enzyme activity. Therefore the individuals with GSTM1 deficiency are not able to detoxify broad specter of xenobiotics and metabolic intermediates, which indicates their increased susceptibility towards various disease development. Indeed, GSTM1-null genotype has been the most studied in terms of higher susceptibility towards various cancers and chronic disease development. Among GST gene polymorphisms, deletion of both GSTM1 alleles represents the most prominent risk factor of ESRD development, although the magnitude of OR (odds ratio) differs in terms of ethnicity of studied population (25–28). Since the kidney is the organ in which bio activation of many xenobiotic usually takes place, it is not surprising that GSTM1 deletion is found to be associated with vulnerable renal parenchyma. Lack of GSTT1 activity is also a result of gene deletion. In a similar manner deletion polymorphism of GSTT1 gene results in increased risk for ESRD development (29), because of inefficient metabolism of many nephrotoxic compounds. Furthermore, the presence of GSTM1-null and GSTT1-null genotypes in combination is able to further increase the ESRD risk (26,28,30).

Regarding SNP polymorphism of GSTP1 gene, it is noted that individuals who carry at least one variant allele (so called GSTP1-low activity genotype), and are therefore with lower GSTP1 activity, are also affected with increased ESRD risk (25,31). A combination of null/low activity GSTM1/GSTT1/GSTP1 genotypes further increases the ESRD risk, as it is shown in few studies, although the magnitude of this association differs significantly (25,28).

Since the alpha class possesses the glutathione dependent peroxidase activity and catalytic efficiency towards many lipid peroxidation intermediates, one could expect that individuals with genetic polymorphism of GSTA1 gene that results in 4-fold lower enzyme activity, would be affected with this polymorphism. However data on the role of GSTA1-low activity genotype are limited. There is only one study in which it was shown no association of GSTA1 polymorphism with higher risk of ESRD development (28).

**GST POLYMORPHISMS ARE ASSOCIATED WITH ENHANCED OXIDATIVE STRESS IN ESRD**

The origin of increased oxidative stress in ESRD patients includes low molecular weight uremic toxins accumulation, loss of antioxidants and bioincompatibility with dialysis membrane (4,32). Besides well established causes of oxidative stress in ESRD, there is an increasing interest in the role of genetic polymorphisms of GSTA1, GSTM1, GSTP1 and GSTT1, in contribution to oxidative damage of proteins and lipids in these patients. Thus, data concerning genetic predisposition to worse oxidative phenotype in patients with ESRD are limited. Recently, it has been shown that individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with GSTM1-null gene variant, having the most pronounced effect. Specifically, malondialdehyde, most commonly used biomarker of lipid peroxidation, has been unambiguously shown to be elevated in ESRD patients carriers of GSTM1-null or GSTT1-null genotype (26,28,33). Moreover, it seems that this deleterious effect of null genotype is even more pronounced when GSTM1 null and GSTT1 null are combined (28). Proteins in plasma of ESRD patients are also oxidatively modified in relation to GST genotypes. It seems that ESRD patients, based on GST genotype, may be stratified regarding carbonyl and nitrosative stress (28). In addition, nucleic acid oxidative damage also occurs in patients on hemodialysis. This kind of damage also depends on GST genotype. Namely, Lin et al. showed that ESRD patients with GSTM1-null genotype have higher plasma level of 8/OH/deoxy guanosine, well established DNA oxidation biomarker (34). The enhancement of oxidative damage associated with low activity or null GST genotypes seems to be biologically plausible, since the up regulation of GST expression and activity observed as reactive phenomenon in uremic milieu (32) cannot reach levels required to enable efficient antioxidant protection of vital macromolecules.
GSTM1 NULL GENOTYPE INFLUENCES CARDIOVASCULAR SURVIVAL IN ESRD

Development of atherosclerosis represents the essential step in cardiovascular complication in ESRD patients which comprises up to 40% of death outcome in this population (3). Results of several studies have revealed the potential role of oxidative stress in atherogenesis (35–37). Since the altered gene expression of GST enzymes influences the level of oxidative damage (28), it is reasonable to assume that it may influence atherosclerotic processes and contribute to cardiovascular complications development. Indeed, the effect of homozygous deletion of GSTM1 gene in lifelong smokers in Netherland has shown a progression of atherosclerosis in these individuals (38). There is an ongoing discussion in the literature whether the incidence of the coronary artery disease is significantly higher in individuals with GSTM1-null and GSTT1-null genotypes (39–42). In addition, in the non-ESRD individuals with GSTM1 and/or GSTT1-null genotypes, higher incidence of cardiovascular diseases has been described (30,43). The latest study of Taspinar et al. has suggested that deletion of both GSTM1 and GSTT1 genes in patients with type 2 diabetes mellitus results in increased susceptibility to advanced atherosclerosis (44). Interestingly, the role of GST polymorphism in fatal cardiovascular diseases development in ESRD patients has been addressed in only two studies. Lin et al., revealed that ESRD patients with the homozygous GSTM1 deletion have higher overall mortality rate when compared to those with the GSTM1-active genotype (34). In another study association between GSTM1-null genotype and specific cardiovascular causes of death, such as myocardial infarction and cerebral vascular insult (CVI) in ESRD has been suggested for the first time (45). The other common GST polymorphisms, such as GSTA1, GSTP1 and GSTT1, showed no association with ESRD patients overall or cardiovascular mortality (45).

FURTHER PERSPECTIVES

GST family members play a dual role in defense mechanisms, which counteract complex biochemical changes present in uremic state. Namely, all products of analyzed GST genes possess both glutathione conjugating and antioxidant enzymatic activity. Results on association of common GST polymorphisms, with oxidative stress development and its possible implications in prognosis, may lead to new approaches in the individualization of antioxidant therapy in ESRD patients.

Figure 1. Possible association between glutathione transferase gene polymorphism and oxidative stress and cardiovascular disease

Literature


